widely known that fatigue is one of the most disabling symptoms in patients with multiple sclerosis (MS) (Krupp et al., 1988) and those with chronic inflammatory demyelinating polyneuropathy (CIDP) (Boukhris et al., 2005; Bissay et al., 2008). Activity-induced fatigue and weakness were also described in not only patients with MS and CIDP but also with multifocal motor neuropathy (Cappelen-Smith et al., 2000; Kaji et al., 2000; Vucic et al., 2010; Straver et al., 2011).

In addition to demyelinating diseases, patients with a neurodegenerative motor neuron disorder often complain of fatigability. Persistent fatigue is a common complaint in patients with amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) (Piepers et al., 2008; McElhiney et al., 2009), and is associated with an impaired quality of life (Robbins et al., 2001). Straver et al. demonstrated that SMA patients had activity-induced weakness more often than normal subjects (Straver et al., 2011). Spinal and bulbar muscular atrophy (SBMA) is also a slowly progressive lower motor neuron disease, and SBMA patients may therefore show fatigability.

Activity-induced fatigue and weakness are caused by repetitive activity or exertion. It has been suggested that this phenomenon is attributable to activity-dependent conduction block (ADCB) arising in demyelinated axons. After repetitive firing, ionic concentration gradients in the axon are restored by increased Na^+/K^+ pump activity (Bostock and Grafe, 1985). Thus, with each pump-cycle, three Na^+ ions are expelled and only two K^+ ions enter (i.e., electrogenic pump), and the axons hyperpolarized by the pump (Schoepfle and Katholi, 1973), resulting in a decrease in the safety factor for impulse transmission. Nerve conduction is blocked if the safety factor is below unity due to leakage of the driving current caused by demyelination. In lower motor neuron disorders, the safety factor could also be reduced at the distal branching points due to collateral sprouting. Therefore, it is possible that ADCB could occur and may contribute to fatigue and weakness in SMA and SBMA patients.

We have developed a novel method to assess axonal activity-dependent hyperpolarization at a constant stimulus frequency using intra-muscular axonal stimulated-single fiber electromyography (s-SFEMG) (Noto et al., 2011). It was shown that tetanic stimulation at a constant rate (5, 10, and 20 Hz) resulted in a significant latency increase in single human motor axons, the extent of which depended on the stimulus frequency. This technique may detect ADCB if the safety factor is significantly reduced by demyelination or increased branching.

Given the recent interest in the mechanism of fatigue and weakness in demyelinating or chronic neurogenic diseases, the aim of this study was to assess the severity of fatigue in patients with SMA or SBMA, and to investigate whether ADCB contributes to fatigue in such patients.

2. Patients and methods

The study was conducted at Chiba University Hospital between October 2009 and March 2011. Informed consent was provided by each subject, and all experiments and the study protocol were conducted in accordance with the Declaration of Helsinki and with the approval of the Ethics Committee of Chiba University School of Medicine for Human Research Studies.

2.1. Subject

The present study included five different patient groups [SMA/SBMA, CIDP, MS, myasthenia gravis (MG), and axonal neuropathy] and normal subjects. The SMA/SBMA group ($n = 22$) consisted of 5 SMA patients and 17 SBMA patients; one of the 5 SMA patients had mutation of the SMN gene and the remaining SMA patients did not have genetic testing and were diagnosed based on the clinical/familial history and electrophysiological examination. All SBMA patients had expanded CAG repeats of the androgen receptor. Disease durations of SMA and SBMA patients were 22.6 ± 9.5 (mean ± SD) years and 10.2 ± 6.4 years, respectively. Modified Rankin Scale scores were 3.2 ± 0.8 and 2.2 ± 0.4.

The CIDP ($n = 16$), MS ($n = 31$), and MG ($n = 33$) groups consisted of consecutive patients in the study period. Neuropathy patients ($n = 20$) consisted of 13 patients with diabetic polyneuropathy, 6 with vasculitic neuropathy and 1 with vitamin B12 deficiency. This study also included 58 normal healthy subjects; none of whom had a neurological disorder, systematic disease, or was taking medication affecting the peripheral nerve function.

s-SFEMG was performed in 21 SMA/SBMA patients, 6 CIDP patients, and 10 normal subjects who consented to the examination protocol.

2.2. Assessment of fatigue

Fatigue was assessed by the Fatigue Severity Scale (FSS) (Krupp et al., 1989). The FSS was developed as a method of evaluating fatigue in patients with MS and other conditions such as systemic lupus erythematosus. The FSS questionnaire is composed of the following 9 statements related to patients' subjective perception of fatigue and its consequences for everyday activities: 1. My motivation is lower when I am fatigued, 2. Exercise brings on my fatigue, 3. I am easily fatigued, 4. Fatigue interferes with my physical functioning, 5. Fatigue causes frequent problems for me, 6. My fatigue prevents sustained physical functioning, 7. Fatigue interferes with carrying out certain duties and responsibilities, 8. Fatigue is among my three most disabling symptoms, 9. Fatigue interferes with my work, family, or social life. Patients are asked to rate their level of agreement (toward 7) or disagreement (toward 0) with the 9 statements. The final score represents the mean value of the 9 items.

2.3. Stimulated-single fiber electromyography

s-SFEMG was performed in the extensor digitorum communis muscle (EDC) using a Nicolet Viking 4 EMG machine (Nicolet Biomedical Japan, Tokyo, Japan), as described previously (Noto et al., 2011). The recordings were made intra-muscularly with a concentric needle electrode (30 G; TECA elite US53153). The high pass filter was set to 2 kHz and the low pass filter to 10 kHz. Intramuscular axonal stimulation was performed with a monopolar needle electrode (28 G; TECA U0809P02) and a reference surface electrode placed 2 cm laterally (Fig. 1). The stimulus duration was 0.1 ms. The distance between the stimulating and recording electrodes was 2 cm. The stimulus intensity was initially determined as 20% above the activation threshold of the target muscle action potential (MAP).

Before this study was performed, we predicted that blockings might occur due to slight movement of either the stimulating or recording electrodes produced by the muscle twitch. To avoid this phenomenon, the fingers of subjects and electrodes were fixed with a strap or a strut as shown in Fig. 1. In fact, during 20-Hz stimulation, the muscle twitch of the EDC muscle was not observed because 20-Hz axonal-stimulation produced persistent contraction of muscle bundles in all subjects. Therefore, the probability of blockings due to the movement of electrodes was low. We also observed the return of a previously blocked muscle action potential after rest in some recordings with blocking. However, we had to wait for over 15 min in each site in order to clear the effect of axonal hyperpolarization (Kiernan et al., 2004), and long time waiting

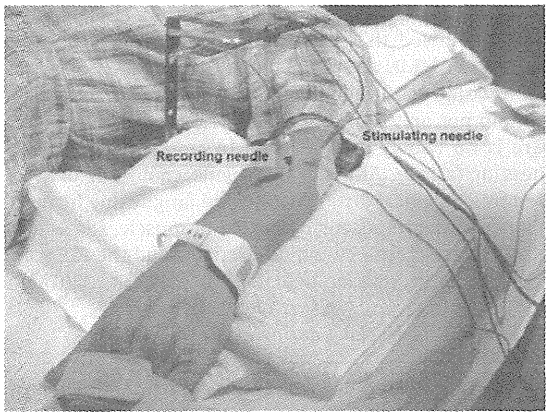

Fig. 1. The set up of stimulated-single fiber electromyography technique. To avoid slight movement of the stimulating and recording electrodes produced by the muscle twitch, fingers of the subject and electrodes were fixed with a strap or a metallic strut.

could not be tolerated for patients. For this reason, the re-stimulation after the rest was omitted.

When intermittent or persistent blockings were observed during stimulation, we increased the stimulus intensity by a further 20% of the previous intensity to secure supra-threshold stimulation. In consideration of tolerability of subjects, the extent of increasing stimulus intensity was up to 20%. A total of 2000 stimuli at 20 Hz were delivered. We examined 5 sites in the EDC in each subject and recorded the latency and shape of some MAPs, and calculated the percentage of MAPs with persistent or intermittent blockings. In this method, we defined persistent blocking as the condition whereby no MAP was evoked during the last 100 stimuli (1901–2000 times). The judgment of blocking was done by latency plotting with a special program for latency measurements (Medical Try System Co. Ltd., Tokyo, Japan).

2.4. Data analysis

The Fisher exact test was used to analyze gender ratio, the differences in proportion of subjects who have high FSS score ($\geqslant$4.0) between groups. The Wilcoxon rank-sum test was used to compare differences in FSS between genders in the normal control group. The correlation between the FSS scores and age in normal controls was tested with Spearman's rank correlation coefficient. Dunnett's multiple comparison tests were applied between normal controls and disease groups. In all comparisons, a p-value of less than 0.05 was considered to be significant. All statistical analyses were performed using STATA software (Stata Corp., Texas, USA) and R software, which is open source and freely available (see http://www.R-project.org).

3. Results

3.1. The fatigue severity scale scores

Clinical profiles and the fatigue severity scale (FSS) scores are shown in Table 1. The mean FSS score was significantly higher in the SMA/SBMA groups than in normal controls. Among the patient groups, the mean FSS score was higher in the SMA/SBMA group than in the other disease groups, although the differences were not significant. There was no significant association of the FSS scores with age or gender. When disabling fatigability was defined as an FSS score of 4.0 or above, as in previous studies (Armutlu et al., 2007; Kaynak et al., 2006), 36% of the normal subjects and 81% of the patients with SMA/SBMA had the degree of fatigue. The percentage of patients with fatigue was higher in the SMA/SBMA group than in the other disease groups.

On analyzing the correlation of the FSS scores with the age, disease duration, or modified Rankin scale score in each disease group, a positive correlation between the FSS scores and disease duration was found only in the MS group.

3.2. Blockings in stimulated-single fiber electromyography

Total numbers of MAPs examined were 41 from 10 normal controls, 85 from 21 SMA/SBMA patients (5 SMA and 16 SBMA patients), and 23 from 6 CIDP patients. The number of examined MAPs per subject ranged from 1 to 9. Table 2 shows the detection rates of intermittent/persistent blocking in normal controls, as well as SMA/SBMA and CIDP patients. Also, in this population, the mean FSS score in the SMA/SBMA group was significantly higher than in normal subjects.

In normal controls, no blocking was observed during 2000 stimuli. A representative recording and the latency scattergraph of a single MAP from a single normal subject are shown in Fig. 2A, 3A, and Supplementary Video 1. The latency prolonged linearly. Although increased jitter with intermittent blockings was found in normal subjects because of subthreshold stimulation, the jitter and blockings disappeared with an increasing stimulus intensity.

In SMA/SBMA and CIDP patients, intermittent or persistent blockings were similarly observed at the 2000th stimulus. We calculated the detection rates of persistent/intermittent blockings at the 2000th stimulus. The percentages of them in SMA/SBMA and CIDP groups were 11.8/45.8 and 13.3/40.0%, respectively (Table 2). Fig. 2B, 3B, and Supplementary Video 2 illustrate a characteristic recording and latency plot of intermittent blocking (finally leading to persistent blocking) from a single SBMA patient. Jitter and blocking were clearly visible in real-time (Supplementary Videos 2, 3). Such a phenomenon was never detected in normal controls. No significant correlation between the FSS score and percentage of intermittent/persistent blockings was demonstrated in SMA/SBMA and CIDP patients.

Table 1
Demographic, clinical findings and fatigue severity scale (FSS) score.

	Normal controls (n = 58)	SMA/SBMA (n = 22)	CIDP (n = 16)	Multiple sclerosis (n = 31)	MG (n = 33)	Neuropathy (n = 20)
Gender (M:F)	28:30	19:3**	13:3*	2:29**	16:17	9:11
Age; mean (range)	50 (20–85)	59 (37–75)	54 (23–79)	45 (20–65)	61 (24–83)**	60 (31–71)**
Disease duration (years); mean (range)	N/A	13.3 (1.5–34.0)	8.7 (0.2–22.0)	10.7 (1.3–38.0)	5.1 (2.0–6.9)	9.7 (0.2–30.0)
Modified Rankin Scale; median (range)	0	2 (2–4)**	2 (1–4)**	2 (1–4)**	1 (0–2)**	1 (1–4)**
FSS score; mean (SD)	3.5 (1.0)	4.9 (1.1)**	4.3 (1.4)	4.3 (1.6)*	4.0 (1.6)	3.6 (1.6)
FSS score $\geqslant$ 4.0 (%)	36	81**	63	65*	61*	35

*P < 0.05, **P < 0.01 vs. Normal control. SMA/SBMA, spinal muscular atrophy/spinal and bulbar muscular atrophy; CIDP, chronic inflammatory demyelinating polyneuropathy; MS, multiple sclerosis; MG, myasthenia gravis; N/A, not applicable.

Table 2
Detection rates of intermittent and persistent blockings during 2000 stimuli at 20 Hz in stimulated-single fiber electromyography and fatigue severity scale score.

	Normal controls	SMA/SBMA	CIDP
	$n = 10$	$n = 21$	$n = 6$
Gender (M:F)	6:4	18:3	5:1
FSS score; mean (SD)	3.8 (1.0)	4.9 (1.1)*	4.3 (1.5)
Intermittent blocking (%)***	0.0 (0.0)	11.8 (16.7)	13.3 (24.2)
Persistent blocking (%)***	0.0 (0.0)	45.8 (32.9)**	40.0 (23.5)*

$P < 0.05$, *$P < 0.01$ vs. Normal control, **mean (SD); SMA/SBMA, spinal muscular atrophy/spinal and bulbar muscular atrophy; CIDP, chronic inflammatory demyelinating polyneuropathy; FSS, fatigue severity scale.

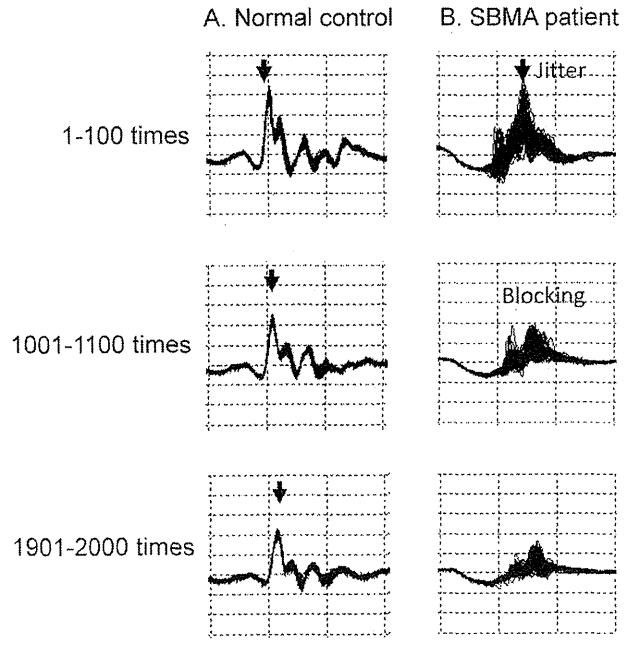

Fig. 2. Examples of superimposed single muscle action potentials during prolonged 20-Hz intramuscular microstimulation in the extensor digitorum communis muscle of a normal control (A) and a spinal and bulbar muscular muscular atrophy (SBMA) patient (B). Traces 1–100, 1001–1100, and 1901–2000 are shown separately. Note a gradual increase in latencies with no block of a muscle action potential (MAP) in a normal control (arrow in A), and jitter and subsequent blocking of a MAP in an SBMA patient (arrow in B).

4. Discussion

The present study demonstrates that patients with SMA/SBMA have prominent muscle fatigue, as well as patients with MS or CIDP. s-SFEMG studies have shown that SMA/SBMA and CIDP patients might have ADCB phenomenon induced by high-frequency axonal stimulation, and that both baseline neuromuscular transmission failure and axonal hyperpolarization could contribute to the activity-dependent changes observed in this study. The pathophysiology and mechanisms of fatigue are different among the disorders. This study firstly shows evidence that ADCB could contribute to muscle fatigue in SMA/SBMA.

4.1. Fatigue in neurodegenerative lower motor neuron disorders

In this study, "fatigue" means activity-induced muscle weakness and fatigability, not baseline muscle weakness and clinical severity. Demyelinating diseases, such as MS, MMN, and CIDP, often cause fatigability (Krupp et al., 1988; Boukhris et al., 2005; Bissay et al., 2008), and ADCB has been regarded as a mechanism of fatigue in MS and MMN (Vucic et al., 2010; Kaji et al., 2000). A recent report showed that SMA patients exhibited fatigue to the same extent as MMN and CIDP patients using FSS (Straver et al., 2011). Our study confirmed that fatigue was a frequently disabling symptom in not only SMA but also SBMA patients.

There is no widely used method to assess activity-dependent weakness or fatigue. Although FSS alone could not discriminate between psychological disinclination and fatigue due to an impairment of nerve, neuromuscular junction or muscle, some questions of FSS are to assess activity-induced fatigue, and the FSS score correlated with motor function deficits (Huisinga et al., 2011).

4.2. Mechanisms for blocking during 20-Hz stimulation

Our s-SFEMG technique detected axonal conduction failure during 20-Hz stimulation in SMA/SBMA and CIDP patients, whereas our method could not identify the site of blocking (axon, neuromuscular junction or muscle) accurately. This was a main limitation of our method. However we considered the main site of conduction failure we observed was axons. The possibility of electrode dislocation cannot be excluded as explanation of disappearance of some potentials. However we think it is possible that the change in the muscle action potential's shape is induced by hyperpolarization of the muscle fiber membrane as Bergmans (2012) emphasized in his recent commentary. This phenomenon was also consistent in this study. The change was generally observed in all subjects, and occurs not at random.

Our previous study demonstrated that tetanic stimulation at 20-Hz results in a significant latency increase in normal subjects (Noto et al., 2011). Along with previous studies, this phenomenon is believed to be induced by activation of the electrogenic Na^+/K^+ pump and resulting axonal hyperpolarization which leads to axonal conduction slowing (Vagg et al., 1998; Kuwabara et al., 2001; Kuwabara et al., 2002; Bergmans, 1970; Lin et al., 2000). Meanwhile, our previous study demonstrated the muscle action potential's shape became smaller and less sharp during stimulation. This phenomenon might be physiological, and was not due to a recording needle dislocation. Bergmans commented to this phenomenon in our previous study that the changes in waveforms and amplitudes in muscle action potentials during stimulation were caused by hyperpolarization of the muscle fiber membrane (Bergmans, 2012).

Regarding the physiological aspects of axonal firing, A 20-Hz stimulation is almost equivalent to physiological maximum voluntary contraction in EDC, and causes axonal hyperpolarization (Burke and Jankelowitz, 2009). This is the reason why we selected 20-Hz stimulation. As seen in Fig. 3B, the latency is gradually prolonged until the blocking occurs, although the dispersion of latencies (i.e., jitter) is also observed simultaneously. These findings may reflect activity-dependent hyperpolarization in the axon and the following blocking (i.e., ADCB).

This technique could not deny that blockings occur at the neuromuscular junction due to dysfunction of the synaptic terminal (e.g., the deficiency of acetylcholine in the axon). 20-Hz s-SFEMG with administration of cholinesterase inhibitor could solve this problem. However, it was difficult to perform it because of invasiveness. A further improvement in methodology is needed in future. In addition, recent studies reported that survival motor neuron protein deficiency produce neuromuscular junction dysfunction (Ling et al., 2012; Martinez et al., 2012). Although this factor might contribute to findings obtained from SMA patients, they were similar to findings in SBMA patients, and were not necessarily specific in SMA patients.

Also, our method was unable to calculate jitter values accurately. For this reason, we judged the intermittent/persistent blocking by latency plotting. Most of the blockings occurred a

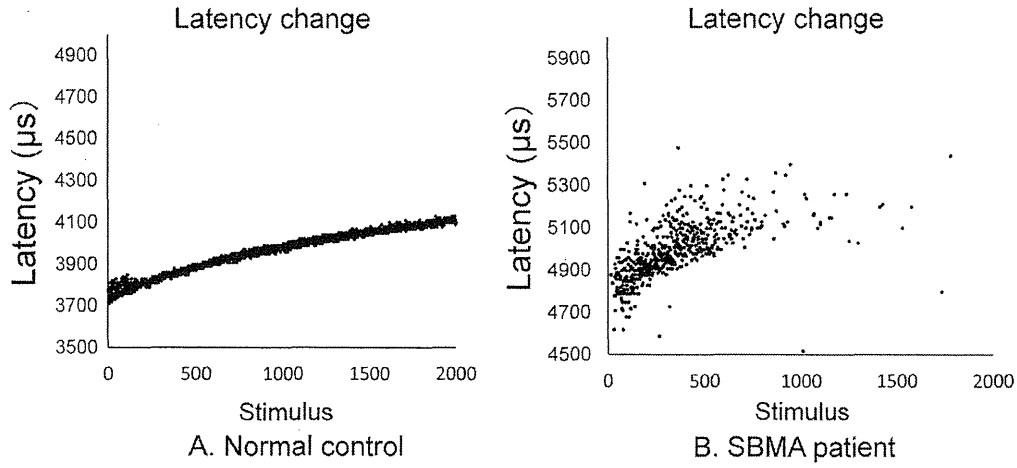

Fig. 3. Examples of the scatter plot of the latencies of single muscle action potentials during 20-Hz stimulation recorded from the extensor digitorum communis muscle of a normal control (A) and a spinal and bulbar muscular atrophy (SBMA) patient (B). The 2 subjects are the same as in Figure 2. Note a progressive increase in latencies during 2000 stimuli at 20 Hz in a normal control, and the dispersion of latencies and subsequent blocking in an SBMA patient.

few seconds after the start of stimulation, and they were judged as absolute blockings by enhancing the stimulus intensity. Enhancing level was set a further 20% of the previous intensity. Although it was difficult to ensure suprathreshold stimulation, the findings of SMA/SBMA and CIDP patients were distinctly different from that of normal controls in our method.

While there were some methodological limitations, blockings observed only during 20-Hz stimulation were likely to suggest conduction failure due to axonal hyperpolarization. Thus, these findings may reflect the existence of ADCB in physiological voluntary activity.

4.3. Activity-dependent conduction block in SMA and SBMA

The mechanism of ADCB in demyelinating neuropathy has been discussed in previous studies (Burke and Jankelowitz, 2009; Park et al., 2011). In SMA and SBMA, the main pathology is slowly progressive axonal loss. ADCB in neurodegenerative lower motor neuron disorders may be related to increased multiple axonal branches associated with collateral sprouting. Computer model by Zhou et al. simulated action potential propagation from a parent myelinated branch through a single branch point to two myelinated daughter branches (Zhou and Chiu, 2001). In the study, action potentials in daughter branches were smaller than that in the parent branch. If an axon has many sprouting branches, an axonal driving current in each nerve terminal branches will be extremely small. Thereby the safety factor could become critically lowered in the nerve terminals. This condition leads to a vulnerability to conduction failure when axons are hyperpolarized. Stålberg et al. reported that paired blocking was seen in ALS and SMA patients using a voluntary-single fiber electromyography method (Stålberg and Thiele, 1972). They proposed that this type of blocking occurred in newly formed sprouts because myelination in new branches was premature and offered a low safety factor for transmission. However, we assume that blocking could also occur in the terminals of many mature branches considering the existence of an axonal hyperpolarization mechanism induced by activity.

This reinnervation mechanism may also partly contribute to ADCB in chronic demyelinating disease, such as CIDP and MMN, because denervation and collateral sprouting are seen in the pathology of these diseases (Bouchard et al., 1999; Van Asseldonk et al., 2003). In addition to reinnervation, axons with higher firing rates might be involved in ADCB in motor neuron disorders. Vucic et al. demonstrated relatively larger increases in the threshold following activity in ALS patients than in healthy controls (Vucic et al., 2007). The central drive for higher firing rates in surviving axons would cause a greater impulse load on the axon also in SMA and SBMA. It is clinically relevant to explore the ionic mechanisms for muscle fatigue, which may provide a new treatment option by modulating the pump activity and specific ionic currents.

Disclosure

Drs. Noto, Misawa, Mori, Kawaguchi, Kanai, Shibuya, Isose, Nasu, Sekiguchi, Beppu, Ohmori, and Nakagawa report no disclosures. Dr. Kuwabara serves as an Associate Editor of *Journal of Neurology, Neurosurgery, and Psychiatry*, and as an Editorial Board Member of *Clinical Neurophysiology*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinph.2012.12.053.

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RESEARCH PAPER

Neck weakness is a potent prognostic factor in sporadic amyotrophic lateral sclerosis patients

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ABSTRACT

Objective To clarify the emergence of muscle weakness in regions of the body that affect survival, and deterioration in activities of daily living (ADL) in amyotrophic lateral sclerosis (ALS) patients.

Methods We conducted a multicentre-based prospective cohort study of patients with ALS. We enrolled 401 sporadic patients with ALS. Death or the introduction of invasive ventilation was defined as the primary endpoint, and the time to five clinical markers of ADL deterioration associated with bulbar paralysis or limb weakness were defined as ADL milestones. Muscle weakness was assessed in the neck flexor muscles; the bilateral abductors of the shoulders; the bilateral wrist extensor muscles; the bilateral flexor muscles of the hips; and the bilateral ankle dorsiflexion muscles. We performed Cox proportional hazards regression analyses for the primary endpoint and the five ADL milestones, adjusting for known covariate prognostic factors for ALS.

Results The Medical Research Council (MRC) score for the neck flexors was the most significant prognostic factor for the primary endpoint (HR 0.74, p<0.001), *loss of speech* (HR 0.66, p<0.001), and *loss of swallowing function* (HR 0.73, p<0.001), and was one of the significant prognostic factors for *loss of upper limb function*, *difficulty turning in bed*, and *loss of walking ability* (p=0.001, 0.002, and 0.008, respectively). The MRC score for the neck flexors was also a significant prognostic factor for covariates of the previously reported prognostic factors.

Conclusions Neck weakness is an independent prognostic factor for survival and deterioration in ADL in Patients with ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterised by progressive upper and lower motor neuron loss, which leads to limb and bulbar paralysis and respiratory failure.[1] Symptoms develop at a progressive rate, and the median survival time from disease onset is 2–4 years.[2–4] However, patients with ALS show extensive variability in clinical courses, with durations ranging from a few months to more than 10 years. Furthermore, major symptoms that differentially affect activities of daily living (ADL) and prognosis also show variability among patients with different disease forms.[5] A better understanding of the factors influencing deterioration in ADL and prognosis would help physicians and patients determine whether and when to introduce non-invasive positive pressure ventilation, tube feeding, tracheostomy and artificial ventilation, and would lead to effective stratification strategies in clinical trials. Several studies have shown that age,[6–10] bulbar symptom onset,[6 7] respiratory function,[3 8 11 12] time from symptom onset to diagnosis,[2 6 10 13 14] functional score[2 14] and rate of disease progression[2 15–17] are predictors of survival. Muscle weakness in particular regions of the body affect the prognosis of ALS, although it has not been sufficiently determined which regions are most predictive.[18] To investigate the longitudinal course of patients with ALS and clarify the emergence of muscle weakness, which affects deterioration in ADL and ALS prognosis, we conducted a prospective, multicentre study.

METHODS

Patient registry and follow-up system

We constructed a multicentre registration and follow-up system called the Japanese Consortium for Amyotrophic Lateral Sclerosis research (JaCALS), which consists of 21 neurology facilities in Japan. Patients with ALS diagnosed in these facilities were consecutively registered with written informed consent. The ethics committees of all the participating institutions approved the study. Full clinical examinations were conducted at registration by neurologists in each of the respective institutions. Muscle strength was manually tested and scored with the scale of the Medical Research Council (six points, range: 0–5)[19] in nine muscle groups as follows: neck flexors; bilateral abductors of shoulders as representatives of proximal upper extremity muscles; wrist extensors muscles as representatives of distal upper extremity muscles; bilateral flexors of hips as representatives of proximal lower extremity muscles; and ankle dorsiflexion muscles as representatives of distal lower extremity muscles. All manual muscle testing was performed with standard positioning and procedures by certified neurologists.[20] The MRC score of the neck

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flexors was determined with the patient in the supine position. We confirmed the inter-rater reliability of the manual muscle testing method employed in this study using 23 patients with neuromuscular disease. The values of the kappa statistics of each muscle ranged from 0.65 to 0.93. To standardise the procedures and the examinations, the three organising doctors (NA, RN, HaW) visited each participating facility and ascertained the evaluation methods for this study.

Disease onset was defined as when the patients became initially aware of muscle weakness or impairment of swallowing, speech, or respiration. We enrolled patients who fulfilled the revised El Escorial criteria.[21] The diagnostic accuracy of the enrolled patients was then assessed by members of the steering committee of the JaCALS. Included patients were then followed-up with telephone surveys conducted by clinical research coordinators (CRC) or with examinations by neurologists every 3 months, and the degree of deterioration in ADL was determined at each time point. We employed the Japanese version of the ALSFRS-R as a scale for ADL, which was validated by Ohashi *et al*, using a telephone survey system.[22] We confirmed the reliability of the telephone survey system for the Japanese version of the ALSFRS-R previously,[23] and the English version of the telephone survey system has been confirmed in several previous studies.[24–26] Prior to the study, we informed and trained the CRCs of the study plan, procedures for the telephone survey, ethical issues relevant to the study, and requisite considerations for patients with ALS and caregivers, and then provided them with a general knowledge of ALS.

We defined a primary endpoint and ADL milestones in the disease course of the patients with ALS and determined their occurrence by telephone survey or examinations by neurologists. The introduction of tracheostomy positive pressure ventilation (TPPV) or death of the patient was defined as the primary endpoint, and TPPV-free survival was defined as survival. *Loss of speech function*, *loss of swallowing function*, *loss of upper limb function*, *difficulty turning in bed*, and *loss of walking ability* were set as ADL milestones. The time at which each ADL milestone occurred was defined as follows: *loss of speech function* was determined to have occurred when non-vocal communication became needed; *loss of swallowing function* was determined to have occurred when parenteral or enteral feeding became needed exclusively; *loss of upper limb function* occurred when the patient needed to be fed and became unable to grip a pen; *difficulty turning in bed* occurred when the patient became unable to turn in bed alone; *loss of walking ability* occurred when the patient became unable to walk without assistance.

Patients

A total of 520 patients with ALS were initially registered in the JaCALS from January 2006 to June 2011. We excluded 26 patients with known gene mutations: 17 patients with SOD-1 mutations, two patients with TDP-43 mutations, two patients with FUS/TLS mutations, three patients with angiogenin mutations, and two patients with C9ORF72 repeat expansions. We also excluded 13 patients with family histories of ALS and 40 patients who were categorised as clinically possible or suspected according to the revised El Escorial criteria. An additional 20 patients for whom we could not obtain follow-up information to their refusal to participate in the telephone survey were also excluded. Twenty patients were excluded due to invalid data. The study finally included 401 sporadic patients with ALS diagnosed as clinically definite, probable, or probable laboratory-supported. Of these, 382 patients were followed for more than a year or died within a year of registration, and 19 patients were censored within a year from registration. Eleven patients declined the telephone survey during the course of the study, and we lost contact with eight patients during the survey.

Statistical analysis

The clinical data of the registered patients were anonymised in each participating facility of the JaCALS and assigned unique patient numbers. The data were then sent to the clinical data centre located at the Nagoya University Graduate School of Medicine and inputted into the JaCALS database.

We performed Cox proportional hazards regression analyses for the time of registration to the primary endpoint or onset of each ADL milestone to evaluate the impact of muscle weakness on the time to the primary endpoint and each decline in ADL. Specifically, for the primary endpoint and each ADL, we evaluated the HR for the MRC scores in nine muscle groups (ie, neck flexors, left and right abductors of shoulders, wrist extensor muscles, flexors of hips and ankle dorsiflexion muscles) at registration, identifying the muscles groups associated with the primary endpoint and five common ADL milestones. Additionally, we examined the HR for each muscle group after adjusting for known prognostic factors as follows: age at registration,[6–10] gender (male vs female),[6 27] disease duration,[2 6 10 13 14] percent vital capacity (%VC),[3 8 11 12] ALSFRS-R score,[14] riluzole use (yes vs no),[28] bulbar symptom,[6 7] and classification according to the revised El Escorial criteria (definite vs probable or probable laboratory-supported).[7 8 10 14] We compared the time from registration to the primary endpoint or each of the previously defined ADL milestones in the patients divided by their degree of muscle weakness using the Kaplan–Meier method. The log-rank test was used to test the null hypothesis that all the Kaplan–Meier curves were equal. A two-sided $p<0.05$ was considered statistically significant. All statistical analyses were conducted using the PASW V.18.0 program (SPSS Inc, Chicago, Illinois, USA).

RESULTS

Demographic characteristics of the registered patients

The patient sample comprised 244 men and 157 women. The median age at disease onset was 62.2 years (IQR: 53.5–68.5 years), and the mean follow-up period was 2.1±1.5 years. The follow-up rate at 1 year after registration was 95.3%. As initial symptoms, 47.4% of the patients showed upper limb weakness, 31.4% lower limb weakness, 22.9% dysarthria, 5.5% dysphagia and 2.0% cervical weakness. At registration, the median score on the ALSFRS-R was 38 (IQR: 32–42). (see online supplementary table S1).

Identification of weakened muscle groups that affect survival and progression to the ADL milestone

Cox proportional hazard regression analyses for the primary endpoint and the ADL milestones

Table 1 shows the results of Cox proportional hazard regression analyses for the primary endpoint and the five ADL milestones, including the MRC scores of the nine muscle groups. The MRC score for the neck flexors was the most significant negative prognostic factor for the primary endpoint, *loss of speech*, and *loss of swallowing function* (HR 0.74, $p<0.001$, HR 0.66, $p<0.001$, HR 0.73, $p<0.001$, respectively). For the *loss of upper limb function*, *difficulty turning in bed* and *loss of walking ability*, the MRC score for the neck flexors was a significant negative prognostic factor (HR 0.77, $p=0.001$, HR 0.77, $p=0.002$, and HR 0.80, $p=0.008$, respectively). Whereas, the MRC score for the left wrist extensors was a significant positive prognostic factor for the primary endpoint and each ADL milestone except for *difficulty turning in bed*.

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Table 1 Multivariate Cox regression analyses for the primary endpoint and each activity of daily living milestone using the MRC score of each muscle group at registration

Muscle group	Primary endpoint		Loss of speech		Loss of swallowing function		Loss of upper limb function		Difficulty turning in bed		Loss of walking ability	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Neck flexors	0.74 (0.65 to 0.86)	<0.001	0.66 (0.56 to 0.76)	<0.001	0.73 (0.63 to 0.85)	<0.001	0.77 (0.66 to 0.89)	0.001	0.77 (0.66 to 0.91)	0.002	0.80 (0.67 to 0.94)	0.008
Left shoulder abductors	0.87 (0.69 to 1.11)	0.266	0.89 (0.69 to 1.14)	0.363	0.89 (0.71 to 1.12)	0.309	0.62 (0.49 to 0.79)	<0.001	0.72 (0.56 to 0.93)	0.012	0.75 (0.57 to 1.00)	0.049
Right shoulder abductors	0.98 (0.77 to 1.25)	0.890	1.11 (0.87 to 1.43)	0.399	1.02 (0.81 to 1.29)	0.867	1.19 (0.94 to 1.50)	0.159	1.08 (0.85 to 1.39)	0.529	0.99 (0.75 to 1.30)	0.917
Left wrist extensors	1.29 (1.04 to 1.59)	0.018	1.28 (1.03 to 1.59)	0.026	1.24 (1.02 to 1.51)	0.034	1.42 (1.14 to 1.77)	0.002	1.24 (1.00 to 1.55)	0.054	1.39 (1.08 to 1.79)	0.010
Right wrist extensors	0.90 (0.74 to 1.08)	0.254	0.88 (0.73 to 1.07)	0.193	0.92 (0.77 to 1.11)	0.380	0.73 (0.60 to 0.88)	0.001	0.80 (0.66 to 0.98)	0.029	0.98 (0.79 to 1.22)	0.884
Left hip flexors	0.99 (0.72 to 1.36)	0.964	0.96 (0.73 to 1.28)	0.791	0.85 (0.62 to 1.15)	0.284	0.74 (0.55 to 0.99)	0.040	0.77 (0.56 to 1.06)	0.115	0.90 (0.61 to 1.32)	0.585
Right hip flexors	0.96 (0.69 to 1.34)	0.830	0.95 (0.70 to 1.28)	0.719	1.09 (0.79 to 1.50)	0.613	1.18 (0.87 to 1.62)	0.290	1.18 (0.84 to 1.66)	0.331	1.06 (0.69 to 1.64)	0.788
Left ankle extensors	1.14 (0.93 to 1.40)	0.214	1.13 (0.94 to 1.34)	0.185	1.14 (0.95 to 1.37)	0.166	1.26 (1.04 to 1.52)	0.021	1.09 (0.91 to 1.30)	0.367	0.93 (0.71 to 1.21)	0.583
Right ankle extensors	0.94 (0.76 to 1.15)	0.530	0.95 (0.79 to 1.14)	0.564	0.94 (0.77 to 1.14)	0.539	0.85 (0.70 to 1.04)	0.125	0.81 (0.68 to 0.97)	0.023	0.72 (0.57 to 0.91)	0.007

According to table 1, the MRC score for the neck flexors was commonly identified as a possible prognostic factor for the primary endpoint and the five ADL milestones. We further examined its impact after adjusting for the other established or potential risk factors, that is, age at registration, gender, disease duration from onset to registration, percent vital capacity (% VC) at registration, ALSFRS-R score at registration, classification according to revised El Escorial criteria, riluzole use and bulbar symptom at registration (table 2). As seen in table 2, the MRC score for the neck flexors was an independent and significant prognostic factor for the primary endpoint, *loss of speech*, *loss of swallowing*, *loss of upper-limb function* and *difficulty turning in bed* in patients with ALS except for *loss of walking ability*. ($p<0.001$, $p=0.001$, $p=0.003$, $p<0.001$, $p=0.027$, respectively). At registration, there were moderate and significant correlations between the MRC score for the neck flexors and the % VC or the ALSFRS-R score. Pearson's correlation coefficients were 0.367 ($p<0.001$) and 0.496 ($p<0.001$), respectively.

Differences in survival time and time to ADL milestones in patients in terms of the MRC score grade for the neck flexors

We divided the registered patients into four categories according to their MRC score for the neck flexors (ie, 5, 4, 3 and ≤2). Figure 1 shows the Kaplan–Meier curves for the four categories for the primary endpoint and each ADL milestone. All the differences between the curves were significant according to a log-rank test ($p<0.001$).

DISCUSSION

In a prospective and multicentre cohort study, we identified that weakness of the neck flexors is a potent factor for the prediction of survival and for the deterioration of ADL, such as speech, swallowing, upper limb function, turning in bed, and walking, in sporadic patients with ALS.

The neck flexors consist of the sternocleidomastoid muscle (SCM), the platysma muscle, hyoid muscle, longus capitis muscle, longus colli and scalenus. These muscles are innervated by motor neurons in the cervical cord (C1–8) and accessory nerve nuclei,[29 30] primarily the C2–4 segments. By contrast, respiratory muscles consist of the diaphragm and the internal and external intercostals muscles, which are innervated by motor neurons of the upper cervical cord (C3–5) and thoracic cord (Th1–Th12), respectively.[30] Thus, the muscles for neck flexion and those for respiration partially share spinal segments of the motor neuron column for their motor innervations. Furthermore, significant correlations are present between compound muscle action potentials of the SCM and those of the diaphragm in patients with ALS,[31] suggesting that neck muscle weakness is correlated with weakness of the diaphragm to some extent in ALS. Because the main cause of death in patients with ALS is respiratory insufficiency, it is reasonable that neck flexor weakness was associated with respiratory impairments and, eventually, survival time. The motor response amplitude of the phrenic nerve motor neurons which are located in the C3–5 segments has been shown to be a significant prognostic factor for survival in patients with ALS.[32] This supports our findings.

Why then is weakness of the neck flexors a determinant factor for the deterioration of ADL for speech, swallowing, upper limb function, truncal turning and walking ability? Recently, some studies have suggested that the degeneration of motor neurons is initially a focal process in ALS that later spreads contiguously throughout the three-dimensional anatomy of connected or neighbouring neurons.[33–36] Dysfunction of speech and swallowing involves the impairment of motor

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Table 2 Multivariate Cox regression analyses with the adjustments of the covariates that we selected for the primary endpoint and each activity of daily living milestone using known predictive factors

	Primary endpoint		Loss of speech		Loss of swallowing function		Loss of upper limb function		Difficulty turning in bed		Loss of walking ability	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
MRC score of neck flexors at registration	0.72 (0.62 to 0.83)	<0.001	0.78 (0.67 to 0.90)	0.001	0.80 (0.69 to 0.93)	0.003	0.76 (0.65 to 0.88)	<0.001	0.83 (0.70 to 0.98)	0.027	0.95 (0.79 to 1.15)	0.601
Age at registration (years)	1.03 (1.02 to 1.04)	<0.001	1.02 (1.01 to 1.03)	0.002	1.03 (1.01 to 1.04)	<0.001	1.01 (0.99 to 1.02)	0.264	1.01 (0.99 to 1.02)	0.265	1.00 (0.98 to 1.01)	0.890
Gender (male vs female)	1.14 (0.85 to 1.52)	0.381	0.85 (0.65 to 1.12)	0.247	1.13 (0.85 to 1.49)	0.398	1.27 (0.97 to 1.68)	0.088	1.01 (0.76 to 1.33)	0.947	0.85 (0.61 to 1.17)	0.309
Duration from onset to registration (years)	0.64 (0.57 to 0.72)	<0.001	0.72 (0.64 to 0.80)	<0.001	0.69 (0.62 to 0.77)	<0.001	0.82 (0.75 to 0.9)	<0.001	0.74 (0.65 to 0.85)	<0.001	0.75 (0.66 to 0.87)	<0.001
%VC at registration	0.98 (0.98 to 0.99)	<0.001	0.98 (0.97 to 0.99)	<0.001	0.98 (0.98 to 0.99)	<0.001	0.99 (0.98 to 1.00)	0.001	0.99 (0.99 to 1.00)	0.007	1.00 (0.99 to 1.00)	0.491
ALSFRS-R at registration	0.97 (0.94 to 0.99)	0.008	0.99 (0.97 to 1.02)	0.483	0.96 (0.93 to 0.98)	0.001	0.96 (0.94 to 0.98)	0.001	0.89 (0.86 to 0.92)	<0.001	0.91 (0.88 to 0.94)	<0.001
El Escorial criteria (probable or probable laboratory-supported)	0.72 (0.53 to 0.99)	0.043	0.61 (0.45 to 0.82)	0.001	0.76 (0.56 to 1.04)	0.087	0.67 (0.50 to 0.90)	0.007	0.71 (0.52 to 0.97)	0.031	0.63 (0.44 to 0.88)	0.008
Riluzole administration	1.02 (0.75 to 1.37)	0.916	1.09 (0.82 to 1.44)	0.551	0.97 (0.73 to 1.29)	0.843	0.95 (0.72 to 1.25)	0.694	0.84 (0.63 to 1.13)	0.258	0.94 (0.68 to 1.31)	0.721
Bulbar symptom at registration	0.91 (0.67 to 1.22)	0.524	2.04 (1.52 to 2.73)	<0.001	1.41 (1.06 to 1.86)	0.018	0.68 (0.50 to 0.93)	0.015	0.63 (0.47 to 0.84)	0.002	0.68 (0.49 to 0.96)	0.028

%VC, percent vital capacity; ALSFRS-R, revised amyotrophic lateral sclerosis functional rating scale.

neurons relayed via the glossopharyngeal, vagus, accessory and hypoglossal nerves to the medulla oblongata.[30] The medulla oblongata and cervical cord motor neurons innervating the neck flexion muscles are anatomically different in their three-dimensional layering, while these two groups of neurons are rather contiguously located. Thus, it may be speculated that if the contiguous spreading of motor neuron degeneration occurs according to the local spreading hypothesis, neck flexion impairment may eventually affect speech and swallowing functions. Furthermore, motor neurons for the neck flexion muscles, which are located in the C1–8 segments,[29 30] are also contiguous or overlapping with those for the upper limb muscles in the C5–Th1 segments, particularly the proximal upper limb muscles.[29 30] Neck flexion and upper limb function may be correlated with disease progression through the local spreading view of motor neuron degeneration. Truncal turning and walking require not only lower limb muscle activities but also power in a broad area of the chest, abdominal and back muscles, which are innervated by the cervical to lumbar cord.[37–39] Therefore, propagation of weakness from the cervical and lumbar areas may affect truncal turning or walking. We need, however, further investigations to demonstrate the underlying mechanisms of the correlation between the neck muscles and other muscles of the body that together determine ADL.

In this study, the MRC score for the left wrist extensors shows a positive prognostic factor for the primary endpoint and some ADL milestones, the reason for which might be that the weakness of the distal muscle in the non-dominant arm was least relevant to survival, or ADL declines so that it was shown to be a positive factor in the multivariate analyses.

A number of studies have demonstrated survival curves for patients with ALS and some factors that influence these survival curves.[18] The majority of these studies have found that older age is a strong risk factor for shorter survival in patients with ALS,[6–10] and the onset of bulbar symptoms is associated with a worse prognosis than the onset of spinal symptoms.[6 7] Several studies have found that a longer diagnostic delay correlates with a better prognosis,[2 6 10 13 14] and that a lower %VC or a percent forced vital capacity (%FVC) is correlated with shorter survival for patients with ALS.[3 8 11 12] The progression rate of the ALSFRS-R at the time of diagnosis was also related to ALS prognosis.[17] Neck flexor weakness has not been listed as a prognostic factor for patients with ALS, and most of these studies evaluated survival alone as an endpoint, and did not determine the onset of loss of speech, swallowing, limb and truncal function. In this study, we showed that neck flexor weakness was not only a novel prognostic factor for survival but also a significant prognostic marker for non-survival events related to ADL decline for patients with ALS.

In the course of ALS, patients must make some difficult decisions, including the use of gastrostomy for tube feeding, the use of assisted ventilation, and end-of-life planning, which require the support of the attending physician and a multidisciplinary team. All patients with ALS should be provided with sufficient information concerning these interventions and given sufficient opportunity for the careful consideration of their decision. In the medical, nursing and social care of patients with ALS, simple and robust indicators for predicting the status of each patient for several months or a year after diagnosis are necessary for patient management. Medical staff and caregivers need to have a predictor of the patient's status in the near future, including survival prognosis and also estimates for the loss of speech, swallowing, limb and truncal function. Our findings may contribute to such prediction.

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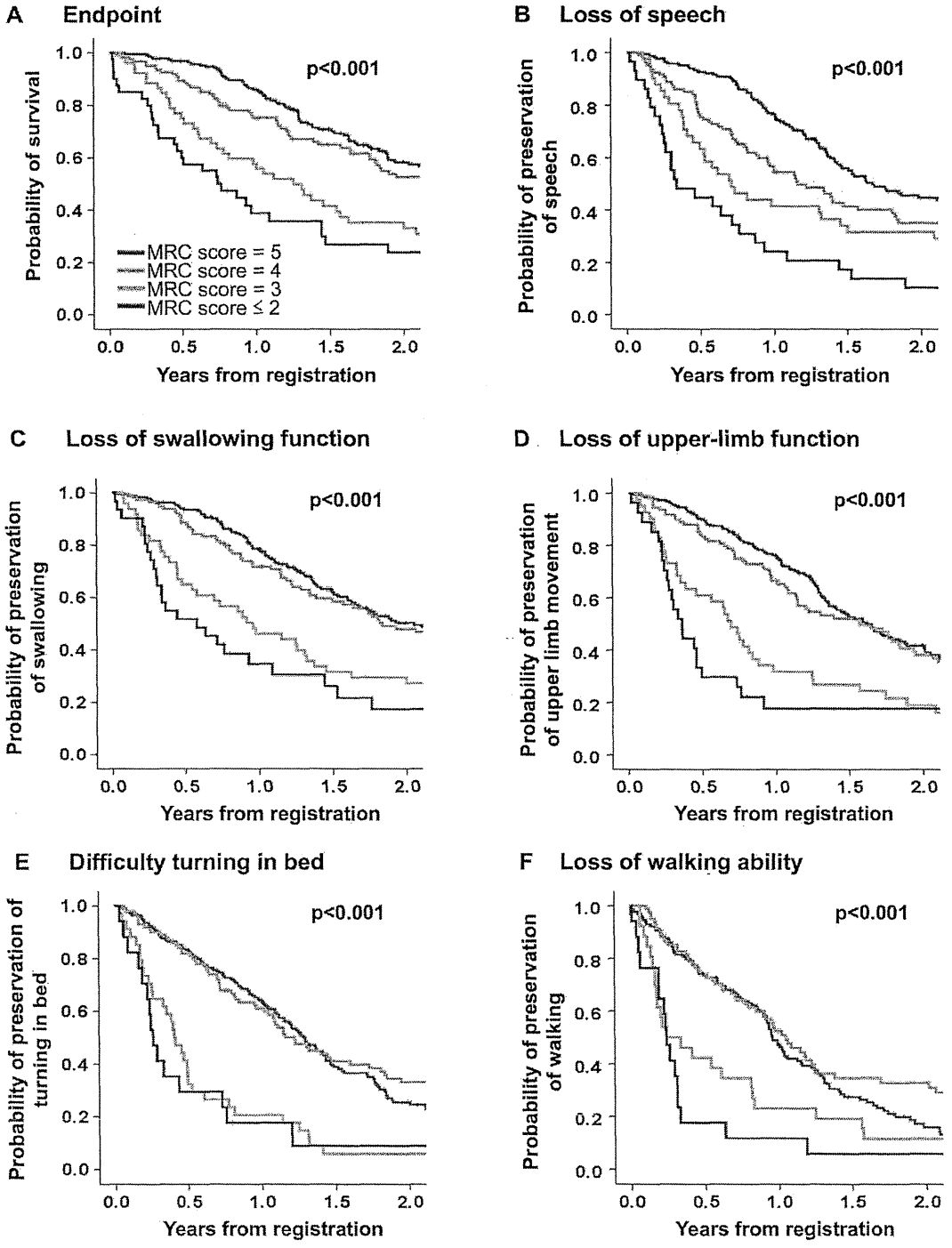

Figure 1 Kaplan–Meier curves according to the MRC score for the neck flexors. The Kaplan–Meier curves for the primary endpoint and each activity of daily living milestone among four categories divided according to the MRC score for the neck flexors were compared by the log-rank test. Curves are shown for the MRC score 5 (blue), the MRC score 4 (green), the MRC score 3 (orange), and the MRC score ≤2 (purple) groups. All the differences of the curves were significant (p<0.001).

The course of ALS is highly variable between patients,[5] which is one of the major factors limiting the power of ALS clinical trials.[40 41] Therefore, robust stratification factors that could divide ALS patient groups depending upon prognosis are needed for designing trials. Compared with known prognostic factors for patients with ALS, such as age, duration from onset to registration, ALSFRS-R at registration, and presence of bulbar symptom, weakness of the neck flexors was a potent and independent prognostic factor. Thus, the MRC score for the neck flexors might be used for stratification factor in a future clinical trial.

Neck extensor muscle weakness with head drop as an early symptom has been reported in a few patients with ALS.[42 43] However, Katz *et al*[44] wrote that neck flexor weakness is typically observed. We assert that neck flexor weakness is commonly observed in patients with ALS, and is useful for the prediction of prognosis.

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The limitations of this study are as follows: registered patients were followed-up by telephone survey, and we did not examine longitudinal changes in the strength of multiple muscles. As we demonstrated, the relationship between the involved muscle groups and survival prognosis and estimates of ADL deterioration would offer insights into the modalities of progression in patients with ALS. However, to examine the pattern of spread more precisely, a cohort study that observes longitudinal changes in the strength of muscle groups and extensions of muscle weakness will be required.

This study analysed a multicentre cohort of patients with ALS in a single nation, Japan. Although the clinical profiles of ALS are broadly similar among countries in previous reports, the outcome of our study would be better confirmed in cohorts of patients with ALS in multiple countries.

In conclusion, we showed that neck weakness is an independent prognostic factor for survival and deterioration in ADL in patients with ALS. We hope that our report will be helpful for clinicians who want to provide medical, social and nursing care to patients with ALS with proper timing, and to researchers as they plan clinical trials for ALS.

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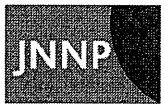

Neck weakness is a potent prognostic factor in sporadic amyotrophic lateral sclerosis patients

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Clinicopathological features of neuropathy associated with lymphoma

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Lymphoma causes various neurological manifestations that might affect any part of the nervous system and occur at any stage of the disease. The peripheral nervous system is one of the major constituents of the neurological involvement of lymphoma. In this study we characterized the clinical, electrophysiological and histopathological features of 32 patients with neuropathy associated with non-Hodgkin's lymphoma that were unrelated to complications resulting from treatment for lymphoma. Nine patients had pathologically-proven neurolymphomatosis with direct invasion of lymphoma cells into the peripheral nervous system. These patients showed lymphomatous cell invasion that was more prominent in the proximal portions of the nerve trunk and that induced demyelination without macrophage invasion and subsequent axonal degeneration in the portion distal from the demyelination site. Six other patients were also considered to have neurolymphomatosis because these patients showed positive signals along the peripheral nerve on fluorodeoxyglucose positron emission tomography imaging. Spontaneous pain can significantly disrupt daily activities, as frequently reported in patients diagnosed with neurolymphomatosis. In contrast, five patients were considered to have paraneoplastic neuropathy because primary peripheral nerve lesions were observed without the invasion of lymphomatous cells, with three patients showing features compatible with chronic inflammatory demyelinating polyneuropathy, one patient showing sensory ganglionopathy, and one patient showing vasculitic neuropathy. Of the other 12 patients, 10 presented with multiple mononeuropathies. These patients showed clinical and electrophysiological features similar to those of neurolymphomatosis rather than paraneoplastic neuropathy. Electrophysiological findings suggestive of demyelination were frequently observed, even in patients with neurolymphomatosis. Eleven of the 32 patients, including five patients with neurolymphomatosis, fulfilled the European Federation of Neurological Societies/Peripheral Nerve Society electrodiagnostic criteria of definite chronic inflammatory demyelinating polyneuropathy. Some of these patients, even those with neurolymphomatosis, responded initially to immunomodulatory treatments, including the administration of intravenous immunoglobulin and steroids. Patients with lymphoma exhibit various neuropathic patterns, but neurolymphomatosis is the major cause of

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neuropathy. Misdiagnoses of neurolymphomatosis as chronic inflammatory demyelinating polyneuropathy are frequent due to a presence of a demyelinating pattern and the initial response to immunomodulatory treatments. The possibility of the concomitance of lymphoma should be considered in various types of neuropathy, even if the diagnostic criteria of chronic inflammatory demyelinating polyneuropathy are met, particularly in patients complaining of pain.

Keywords: chronic inflammatory demyelinating polyneuropathy; demyelination; lymphoma; neurolymphomatosis; neuropathy

Abbreviations: CIDP = chronic inflammatory demyelinating polyneuropathy; EFNS/PNS = European Federation of Neurological Societies/Peripheral Nerve Society; FDG = fluorodeoxyglucose; IVIg = intravenous immunoglobulin

Introduction

Lymphoma causes various neurological manifestations that might affect any part of the nervous system and occur at any stage of the disease (MacKintosh *et al.*, 1982; Giglio and Gilbert, 2006; Antoine and Camdessanché, 2007; Briani *et al.*, 2011; Baehring and Batchelor, 2012). Involvement of the PNS is one of the major constituents of the neurological disorders associated with lymphoma (Walsh, 1971), occurring in 5% of patients with lymphoma (Hughes *et al.*, 1994).

In addition to the direct invasion of lymphoma cells into the PNS (Baehring *et al.*, 2003; Grisariu *et al.*, 2010; Baehring and Batchelor, 2012), the causes of peripheral neuropathy in lymphoma include chemotherapy, radiation therapy, stem cell transplantation, malnutrition, infection, hyperviscosity, secondary amyloidosis, compression, and paraneoplastic syndrome (Correale *et al.*, 1991; Koike *et al.*, 2011*a*). The definitive diagnosis of neuropathy in patients with lymphoma enables the establishment of an appropriate treatment strategy for these patients. For patients not yet diagnosed with lymphoma, the appropriate diagnosis of neuropathy is also important because it enables the treatment for lymphoma to be initiated at an early stage of the disease. However, the conclusions regarding the clinical, electrophysiological and pathological features of neuropathy associated with lymphoma are not always definitive and have not enabled a detailed understanding of the pathophysiology of this condition. The types of neuropathy might include neuropathy associated with the infiltration of lymphoma cells, known as neurolymphomatosis; demyelinating neuropathy mimicking Guillain-Barré syndrome or chronic inflammatory demyelinating polyneuropathy (CIDP); sensory neuronopathy; vasculitic neuropathy; and neuropathy associated with paraproteinaemia (Bosch *et al.*, 2005; Kelly and Karcher, 2005; Viala *et al.*, 2008; Briani *et al.*, 2011). According to a previous study, polyneuropathy with demyelinating features was the most common form of neuropathy associated with lymphoma, and humoral factors might play an important role in this disease (Viala *et al.*, 2008). However, reports have also described the presence of demyelination in neurolymphomatosis (Kohut, 1946; Moore and Oda, 1962). Hence, the clinicopathological features of neuropathy associated with lymphoma, particularly the correlation of these features with lymphomatous cell invasion or the paraneoplastic condition, and their prognostic features have not yet been well characterized.

In the present study, we assessed the clinical, electrophysiological and histopathological findings of 32 patients with neuropathy associated with lymphoma and elucidated the clinicopathological features of neuropathy in these patients.

Materials and methods

Patients

We retrospectively investigated patients with neuropathy associated with lymphoma who were referred to the neurological department of our institutions from 1997 to 2012. The diagnosis of lymphoma was pathologically confirmed based on the 2001 and 2008 World Health Organization classification or the Revised European-American Lymphoma classification in all patients (Harris *et al.*, 1994; Campo *et al.*, 2011). The patients underwent clinical and neurological assessments, routine blood and urine studies, and CSF analysis. Neurological examinations were performed repetitively by at least two neurologists in each case. The presence of neuropathy was clinically defined through the presence of sensory and/or motor signs and the reduction or absence of deep tendon reflexes without pathological reflexes. Nerve conduction studies were used to confirm the presence of neuropathy in each case. Values that deviated from the mean ± two standard deviations (SD) of these controls were defined as abnormal. The muscle strength was assessed through manual muscle testing. Sensory examinations were performed to evaluate pinprick, temperature, light touch, vibratory, and joint position sensations. Autonomic involvement was characterized in terms of the abdominal, urinary, and orthostatic symptoms; pupillary responses; and sweating.

A detailed history of illness was obtained from each patient and the patient's family concerning the lifestyle, occupation, diet and amount of alcohol consumed daily. Patients with underlying diseases other than malignant lymphoma that might cause neuropathy, such as diabetes mellitus, renal failure, vitamin deficiency, thyroid dysfunction, cachexia, and autoimmune disease, were excluded from the study. Patients with Waldenström's macroglobulinaemia were excluded. Patients who were considered to have had treatment-induced neuropathy were also excluded (Windebank and Grisold, 2008; Koike *et al.*, 2011*a*). We excluded cases exhibiting neurological onset within 1 month of the initiation of treatment to exclude neuropathy associated with the side effects of chemotherapy or radiation. Clinical and pathological findings in one patient (Patient 19) were reported in a previous study (Kobayashi *et al.*, 2005). Informed consent was obtained from all patients. The study was approved through the Ethics Committee of Nagoya University Graduate School of Medicine.

Electrophysiological assessment

A nerve conduction study was performed on all patients. Motor and sensory conduction were measured in the median, ulnar, tibial and

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sural nerves using a standard method with surface electrodes for stimulation and recording (Koike *et al.*, 2005, 2008*a*; Suzuki *et al.*, 2008). Motor conduction was investigated in the median, ulnar and tibial nerves through recordings obtained from the abductor pollicis brevis, abductor digiti minimi, and abductor hallucis brevis, respectively. The following nerve segments were used to calculate the motor conduction velocity: wrist to elbow for the median nerve, wrist to distally at the elbow for the ulnar nerve, and ankle to popliteal fossa for the tibial nerve. The sensory conduction was investigated in the median, ulnar and sural nerves using antidromic recordings from ring electrodes at the second and fifth digits for the median and ulnar nerves, respectively, and bar electrodes at the ankle for the sural nerve. The sensory conduction velocity was calculated for the distal segment. The amplitudes of the compound muscle action potential and sensory nerve action potential were measured from the baseline to the first negative peak. Waveforms were also analysed to assess temporal dispersion. A conduction block was defined according to the electrodiagnostic criteria of the European Federation of Neurological Societies/Peripheral Nerve Society (EFNS/PNS) (Joint Task Force of the EFNS and the PNS, 2010). The normal control values were based on previously published reports (Koike *et al.*, 2005; Suzuki *et al.*, 2008).

Pathological assessment

Details of the pathological assessments are described in Supplementary material. A sural nerve biopsy was performed in 20 patients as previously described (Sobue *et al.*, 1989; Koike *et al.*, 2003, 2010). The specimens were divided into two portions. The first portion was fixed in 2.5% glutaraldehyde in a 0.125 M cacodylate buffer (pH 7.4) and embedded in epoxy resin. The density of the myelinated fibres was assessed in toluidine blue-stained semi-thin sections as previously described (Sobue *et al.*, 1990*a*; Koike *et al.*, 2001, 2004). The density of unmyelinated fibres was assessed in uranyl acetate- and lead citrate-stained ultra-thin transverse sections as previously described (Koike *et al.*, 2003, 2007, 2008*b*). A fraction of the glutaraldehyde-fixed sample was processed for a teased-fibre study (Sobue *et al.*, 1989; Dyck *et al.*, 2005). The control values were based on a previous report (Koike *et al.*, 2008*b*).

The second portion of the specimen was fixed in a 10% formalin solution and embedded in paraffin. Sections were cut using routine methods and stained with haematoxylin and eosin and Congo red. Immunohistochemical assessments were performed using the peroxidase–antiperoxidase method in consecutive deparaffinized sections (Sobue *et al.*, 1990*b*; Asano *et al.*, 2005, 2006).

An autopsy was performed on five patients (Patients 1, 2, 7, 8 and 19) as previously described (Sobue *et al.*, 1989, 1990*a*; Koike *et al.*, 2011*b*).

Treatment and assessment of the response to the treatment

The functional status of the patients was assessed at the peak phase according to the modified Rankin scale (van Swieten *et al.*, 1988): 0 = no symptoms at all; 1 = no significant disability despite the presence of symptoms, demonstrated as the ability to perform all typical duties and activities; 2 = slight disability, demonstrated as the inability to perform all previous activities but the ability to perform self-care without assistance; 3 = moderate disability, demonstrated as requiring some help but being able to walk without assistance; 4 = moderately severe disability, demonstrated as the inability to walk without assistance and to attend to bodily needs without assistance; and 5 = severe disability, demonstrated as being bedridden and incontinent and requiring constant nursing care and attention.

The response to the treatment for neurological symptoms was defined using the following terms: effective (2+), i.e. upgraded according to the modified Rankin scale after treatment, and mildly effective (1+), i.e. a reduction in the neurological symptoms without an upgrade on the modified Rankin scale after treatment.

Statistical analyses

The quantitative data are presented as the means ± SD. The statistical analyses were performed using the χ^2 test, Mann-Whitney *U*-test, or Spearman's rank correlation analysis, as appropriate. $P < 0.05$ was considered to indicate significance.

Results

Background and laboratory features

The background and laboratory features are shown in Table 1. The patient cohort included 21 males and 11 females. The age at neuropathy onset was 64.9 ± 13.1 years and ranged from 30–86 years. All patients had non-Hodgkin's lymphoma. Twenty-six patients had B cell lymphoma, and six patients had T cell lymphoma (Patients 5, 7, 16, 18, 31 and 32). In the patients with B cell lymphoma, the most common type was diffuse large B cell lymphoma, observed in 20 of 26 patients. The age at onset was significantly older in patients with B cell lymphoma than in those with T cell lymphoma (68.1 ± 11.1 versus 51.0 ± 12.9 years, respectively, $P < 0.01$). Twenty-three of the 32 patients manifested a focality of the distribution of neuropathic symptoms, as indicated by a multifocal mononeuropathic pattern in the extremities and/or unilateral cranial nerve involvement, whereas the other nine patients were characterized as having a symmetrical polyneuropathy pattern (Fig. 1, Table 2 and Supplementary Fig. 1). Fifty-five per cent of the patients (14 patients with B cell lymphoma and three patients with T cell lymphoma) were referred for the first time because of neuropathic symptoms, and the presence of lymphoma was not diagnosed at the time of the first referral. The other 45% (11 patients with B cell lymphoma and three patients with T cell lymphoma) were diagnosed as having lymphoma before the neurological symptoms appeared, and the duration from the appearance of lymphoma to that of neuropathy was 41.3 ± 37.7 months. Thirteen of these patients had received chemotherapy, and the chemotherapy was finished within 1 year of the diagnosis of lymphoma, with no neurological symptoms at the time of the cessation of chemotherapy.

An abnormal elevation of the serum soluble interleukin 2 receptor was observed in 17 of the 24 (71%) examined patients (range 343–30 500 U/ml; mean ± SD, 2868 ± 6128; normal 220–530). The CSF was examined in 29 patients, and an elevated cell count was observed in 12 patients (range 0–318/mm^3; mean ± SD, 21.0 ± 59.9), whereas protein abnormality was observed in 20 patients (range 26–466 mg/dl; mean ± SD, 116 ± 99). These values were not significantly different between the patients with B cell lymphoma and those with T cell lymphoma. The cytology of the CSF revealed mononuclear cells with an atypical nuclear appearance

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Table 1 Clinical features and laboratory data of neuropathy associated with lymphoma

Patients	Sex	Age	Type of lymphoma	Duration of lymphoma until neuropathic onset (months)	Serum sIL-2R (U/ml)*	CSF findings**			FDG-PET
						Cell (no./mm^3)	Protein (mg/dl)	Cytology	
Neurolymphomatosis***									
1	M	56	DLBCL	−12	343	1	47	−	ND
2	F	73	DLBCL	1	7020	1	30	+	ND
3	F	45	DLBCL	−11	ND	25	124	+	ND
4	M	70	DLBCL	29	467	1	125	−	ND
5	M	56	T cell	−9	ND	318	166	+	ND
6	M	64	DLBCL	−7	1860	ND	ND	ND	ND
7	M	51	ATLL	−20	1590	1	35	−	ND
8	M	69	BL	1	529	0	94	−	ND
9	F	62	DLBCL	5	924	1	65	−	Negative
10	F	82	DLBCL	72	509	1	30	−	Brachial plexus
11	F	75	DLBCL	12	1009	2	30	−	Brachial plexus, adrenal gland, abdominal muscle
12	F	75	DLBCL	6	ND	0	55	−	Lumbar plexus
13	M	63	B cell	−6	441	42	328	+	Lumbar plexus
14	M	76	DLBCL	−12	356	16	34	−	Brachial plexus, abdominal lymph node
15	M	58	DLBCL	−12	915	3	39	−	Brachial plexus
Paraneoplastic neuropathy									
CIDP-type									
16	M	65	AILT	44	ND	4	120	−	ND
17	M	61	LPL	−12	635	1	191	−	Iliopsoas muscle
18	M	61	MF	132	ND	ND	ND	ND	ND
Sensory ganglionopathy									
19	M	63	DLBCL	−72	1200	25	116	−	ND
Vasculitic neuropathy									
20	F	74	DLBCL	24	839	0	26	−	ND
Unclassified group									
Multiple mononeuropathy									
21	M	42	DLBCL	−20	558	0	35	−	Testis
22	M	55	DLBCL	−3	1630	6	153	ND	ND
23	M	86	DLBCL	−2	ND	5	145	−	ND
24	M	73	DLBCL	−6	2350	2	33	−	ND
25	F	78	FL	65	2410	1	133	−	Negative
26	M	72	LPL	51	4230	25	110	−	ND
27	F	84	DLBCL	76	ND	ND	ND	ND	ND
28	F	80	DLBCL	−3	2548	52	212	+	ND
29	M	69	DLBCL	−6	5491	10	112	−	Adrenal gland
30	M	66	MCL	ND	ND	6	226	−	ND
Polyneuropathy									
31	F	30	PTCL-U	−21	484	45	466	−	ND
32	M	43	T cell	60	30500	14	94	+	Bone

ATLL = adult T cell leukaemia/lymphoma; AITL = angio-immunoblastic T cell lymphoma; B cell = unclassifiable B cell lymphoma; BL = Burkitt lymphoma; DLBCL = diffuse large B cell lymphoma; FDG = fluorodeoxyglucose; FL = follicular lymphoma; LPL = lymphoplasmacytic lymphoma; MCL = mantle cell lymphoma; MF = mycosis fungoides; ND = not determined; PTCL-U = peripheral T cell lymphoma unspecified; sIL-2R = soluble interleukin-2 receptor; T cell = unclassifiable T cell lymphoma; + = positive − = negative.

*Normal range, 220-530 U/ml.

**The level of protein and cell count were those at the first examination. The cytology was performed twice in Patients 1, 13 (positive at second examination). and 19, three times in Patients 2 (positive at the first examination), 24, and 31, and 14 times in Patient 32 (positive at the first examination). Flow cytometry was. performed in Patients 5, 13, 28 and 32, and revealed the findings corresponding to each specific diagnosis of lymphoma.

***Patients 1 to 9 were pathologically-proven neurolymphomatosis, whereas Patients 10 to 15 were FDG-PET assessed neurolymphomatosis.

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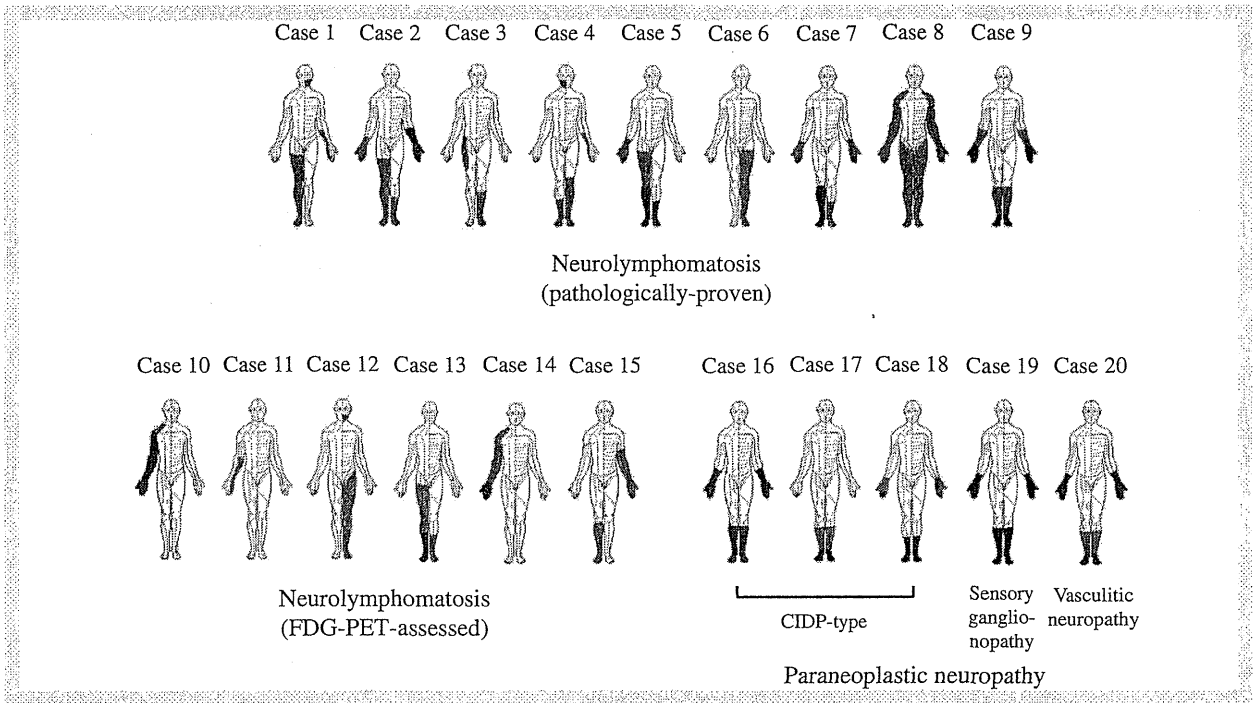

Figure 1 Distribution of sensory deficits in representative cases. Cases 1 to 9 and 10 to 15 represent pathologically-proven and FDG-PET-assessed neurolymphomatosis, respectively. All cases in this category, except for Cases 8 and 9, showed multiple mononeuropathy patterns, whereas Cases 8 and 9 manifested a symmetrical polyneuropathy pattern. Cases 16 to 20 were pathologically-proven paraneoplastic neuropathy. All patients manifested a symmetrical polyneuropathy pattern. Cases 16 to 18 were diagnosed as chronic inflammatory demyelinating polyneuropathy-type neuropathy, Cases 19 was diagnosed as sensory ganglionopathy, and Case 20 was diagnosed as vasculitic neuropathy. The red areas represent those of mild to moderate sensory deficits, while the black areas are where there is severe sensory deficits.

in six of the 28 examined patients. Onconeural antibodies were screened in seven patients (Patients 3, 7, 8, 15, 16, 20 and 31), and anti-Hu, anti-Ri, ANNA-3, anti-Yo, anti-PCA-2, anti-PCA-Tr, anti-CV2, amphiphysin, anti-striatal, anti-P/Q type calcium channel, N-type calcium channel, and anti-ganglionic acetylcholine receptor antibodies were negative in all seven patients. The sera of these patients were assessed based on an indirect immunofluorescence assay at Mayo Medical Laboratories. In Patient 19, the serum IgM antibody against GD1b was positive (× 1000) in an ELISA. Whole-body fluorodeoxyglucose (FDG)-PET was performed in 12 patients, and lymphoma was detected in five of six patients before the clinical discovery of lymphoma (Table 1).

Classification of neuropathy

Neuropathy associated with lymphoma can be classified broadly into neurolymphomatosis, which represents the direct invasion of lymphoma cells into the PNS, and paraneoplastic neuropathy, which represents damage remote from the site of lymphoma (Koike *et al.*, 2011a). Nine patients (Patients 1–9) were considered to have pathologically-proven neurolymphomatosis with lymphomatous cell invasion into the PNS. The direct invasion of lymphoma cells into the PNS was confirmed through biopsy or autopsy (Figs 3 and 4; Supplementary Tables 1 and 2). Additionally, six other patients (Patients 10–15) were considered to have neurolymphomatosis based on the FDG-PET study. FDG accumulation along the peripheral nerves was detected using PET, and the results strongly suggested the presence of neurolymphomatosis in these patients (Fig. 2A and Supplementary Fig. 2).

Five patients (Patients 16–20) were considered to have paraneoplastic aetiologies. These patients exhibited pathological findings suggesting CIDP, sensory ganglionopathy, or vasculitic neuropathy without lymphomatous cell invasion (Supplementary Tables 1 and 2). All five of these patients exhibited a symmetrical polyneuropathic pattern with respect to symptom manifestation (Fig. 1, Table 2 and Supplementary Fig. 1). Three patients (Patients 16–18) manifested subacute to chronic progressive, sensorimotor symmetrical polyneuropathy. The sural nerve biopsy specimens revealed extensive segmental demyelination without lymphomatous cell invasion in these patients (Supplementary Table 1). Another patient (Patient 19) manifested sensory ganglionopathy as previously described (Kobayashi *et al.*, 2005). The autopsy revealed a loss of neurons in the dorsal root ganglia with the preservation of motor neurons in the spinal cord. Patient 20 exhibited vasculitis that was pathologically confirmed through a sural nerve biopsy specimen, although the patient manifested a symmetrical polyneuropathy pattern. Vasculitis in this patient was observed in the epineurial blood vessels, and the infiltrating cells did not present an atypical appearance with a mixture of CD3- and CD20-positive cells, suggesting that the vasculitis was

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Table 2 **Neuropathic features of neuropathy associated with lymphoma**

Patients	Progression*	Type of neuropathy	Cranial nerve involvement	Muscle weakness	Sensory disturbance		Spontaneous pain	Autonomic failure	EFNS/PNS CIDP electropiagnostic criteria**
					Superficial sensation	Deep sensation			
Neurolymphomatosis***									
1	Subacute	MM	V	3+	2+	2+	3+	–	Possible
2	Subacute	MM	VI	2+	1+	3+	3+	–	
3	Chronic	MM	III VI VII	3+	2+	0	1+	Adie pupil	Possible
4	Subacute	MM	V VII	3+	2+	2+	–	–	Definite
5	Chronic	MM	–	2+	2+	3+	–	–	Possible
6	Chronic	MM	–	3+	1+	0	3+	–	
7	Chronic	MM	XII	3+	3+	2+	1+	–	Possible
8	Subacute	PN	–	1+	2+	3+	–	–	Definite
9	Chronic	PN	–	3+	3+	3+	1+	–	Definite
10	Chronic	MM	–	3+	3+	3+	2+	–	Definite
11	Chronic	MM	–	3+	1+	1+	1+	–	Probable
12	Chronic	MM	V	3+	2+	0	1+	–	
13	Subacute	MM	VII	3+	2+	3+	3+	–	
14	Chronic	MM	–	3+	3+	3+	2+	–	
15	Chronic	MM	VII	3+	2+	1+	3+	–	Definite
Paraneoplastic neuropathy									
CIDP-type									
16	Subacute	PN	–	3+	1+	3+	–	–	Possible
17	Chronic	PN	–	3+	1+	3+	–	–	Definite
18	Subacute	PN	–	3+	3+	3+	–	–	Definite
Sensory ganglionopathy									
19	Chronic	PN	–	1+	1+	3+	–	–	Definite
Vasculitic neuropathy									
20	Subacute	PN	–	2+	3+	3+	1+	–	
Unclassified									
Multiple mononeuropathy									
21	Chronic	MM	–	3+	1+	1+	3+	–	Possible
22	Subacute	MM	X	3+	3+	2+	1+	–	Definite
23	Subacute	MM	–	3+	1+	2+	–	–	
24	Chronic	MM	IX X XII	3+	2+	2+	2+	–	Definite
25	Subacute	MM	–	2+	2+	3+	–	–	Definite
26	Chronic	MM	–	1+	1+	3+	1+	–	Possible
27	Subacute	MM	–	3+	3+	3+	–	–	
28	Subacute	MM	VI VII IX	3+	0	2+	–	–	
29	Chronic	MM	VII IX	3+	1+	2+	1+	–	
30	Acute	MM	III IV VI VII IX	3+	0	3+	–	–	Possible
Polyneuropathy									
31	Acute	PN	–	2+	0	3+	1+	–	Possible
32	Acute	PN	–	2+	0	1+	–	–	

MM = multiple mononeuropathy; PN = polyneuropathy; III = oculomotor nerve; IV = trochlear nerve; V = trigeminal nerve; VI = abducens nerve; VII = facial nerve. IX = glossopharyngeal nerve; X = vagus nerve; XII = hypoglossal nerve; 0 = absent; + = present.
1+, 2+, 3+ represent minimal, moderate, and severe involvement for muscle weakness and sensory disturbance.
*Acute = within 4 weeks; subacute = 4 weeks to 3 months; chronic = more than 3 months.
**Based on the European Federation of Neurological Societies/Peripheral Nerve Society Guideline on management of.
chronic inflammatory demyelinating polyradiculoneuropathy (Joint Task Force of the EFNS and the PNS, 2010).
***Patients 1 to 9 were pathologically-proven neurolymphomatosis, whereas Patients 10 to 15 were fluorodeoxyglucose-PET assessed neurolymphomatosis.

not caused by lymphomatous cells. These forms of neuropathies have been described as paraneoplastic neuropathies (Graus *et al.*, 2004; Koike *et al.*, 2011*a*), and thus, these patients were considered as having 'paraneoplastic neuropathy'.

The other 12 patients were not specifically classified as having neurolymphomatosis or paraneoplastic neuropathy, as the diagnostic pathological or radiological findings described above were not obtained. These patients were assigned to an 'unclassified' group. Ten of these patients (Patients 21–30) exhibited multiple mononeuropathy, whereas the other two patients (Patients 31 and 32) exhibited signs of symmetrical polyneuropathy (Supplementary Fig. 1).

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